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Advisory Committee for Pharmaceutical Science

Briefing Document

Levothyroxine Bioequivalence

Advisory Committee Meeting – 12-13 March 2003

THIS DOCUMENT CONTAINS FULLY RELEASABLE INFORMATION

Abbott Laboratories

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1.0 Executive Summary

The purpose of this document, and Abbott's participation in the March 13, 2003 Pharmaceutical Science Advisory Committee meeting, is to identify and discuss scientific issues related to the bioequivalence assessment criteria of levothyroxine sodium (LT_4) products.

Levothyroxine or thyroxine (T_4) is an endogenous hormone secreted from the thyroid gland and is subject to complex biologic regulation. As such, it has characteristics different from drugs for which there are no endogenous levels. Exogenously administered LT_4 hormone is indistinguishable from endogenously secreted T_4, both in its physiologic effects and its quantification as measured in blood. The current FDA guidance for assessment of bioequivalence of administered LT_4 products does not take into account the contribution of endogenous T_4. The presence of endogenous T_4 and its dynamic regulation confound the assessment of bioequivalence of LT_4 products in healthy normal subjects, and consequently, preclude any conclusions about their therapeutic substitution in patients.

We describe a recent study conducted by Abbott Laboratories that highlights these issues with the current FDA Guidance for assessing bioequivalence of LT_4 products. The study demonstrates that, following the current FDA criterion for levothyroxine sodium products, the use of T_4 pharmacokinetic parameters uncorrected for endogenous T_4 would result in declaring two products bioequivalent when they actually differ in drug content by as much as 33%. Considering the margin by which the conditions for declaring bioequivalence were passed in this study, products that differ by even more than 33% would also have a high likelihood of being declared bioequivalent. Three methods of correction for endogenous T_4 levels were evaluated, but none of the methods could discern products that differ by 12.5%; dosage changes of such magnitude are clinically important.

The clinical relevance of a 12.5% difference in dose is substantiated by product labeling, standard medical management of thyroid patients, and data from clinical studies. In class labeling for all LT_4 products, it is recommended that titration be done in 12.5 to 25 μg increments for elderly patients with cardiac disease; who represent a significant number of the 13 million LT_4-treated patients in the U.S. In fact, the FDA has explicitly recognized the clinical relevance of these dosage increments particularly with respect to patient safety. In addition, physicians employ a large number of dosage strengths to effectively titrate patients to normal thyroid status, which generally requires a dose between 100 and 150 µg. In this dose titration process, 12-13 µg is the second most commonly used dose increment or decrement. Finally, mildly abnormal thyroid function, which may result from slight under- or over-dosing, has been demonstrated in clinical studies to have adverse effects on fetal development, lipids, and cardiovascular disease. For patients with thyroid cancer there is an extra concern, in that slight under-treatment increases the risk of cancer recurrence and metastatic disease.

Careful consideration should be given to developing a specific guidance for the assessment of bioequivalence of levothyroxine sodium products. This guidance must adequately consider the unique nature of the thyroid hormone system and the demonstrated limitations of the current guidance not adequately remedied by simple methods for baseline correction.

2.0 Thyroid Biology

Thyroxine (T_4) is an endogenous molecule that is synthesized and released from the thyroid gland in response to thyrotropin or thyroid-stimulating hormone (TSH) (Figure 1). T_4 is a "pro-hormone" that is converted via deiodination in tissues to triiodothyronine (T_3), the most biologically potent form of thyroid hormone. Thyroid hormones (T_4 and T_3) affect protein, lipid, and carbohydrate metabolism, growth, and development. They stimulate the oxygen consumption of most cells of the body, resulting in increased energy expenditure and heat production, and possess a cardiac stimulatory effect that may be the result of a direct action on the heart. Thyroid hormones, T_4 and T_3, are specifically bound by three different plasma transport proteins, each with its specific affinity and capacity for T_4 and T_3. T_3 controls the transcription of numerous genes that are vital to growth and development. With thyroid hormone receptors in virtually every tissue in the body, thyroid hormone affects, via control of specific genes, proper brain development (myelin basic protein gene) and growth (growth hormone gene), and muscle function (myosin heavy chain gene, sarcoplasmic reticulum ATPase gene) and cholesterol levels (LDL-receptor gene).[1,2]

The thyroid hormone system is under the tight feedback regulation by the hypothalamic-pituitary axis, which senses the levels of T_3 and T_4, and modulates the release of hypothalamic thyrotropin-releasing hormone (TRH) and pituitary TSH (Figure 1).

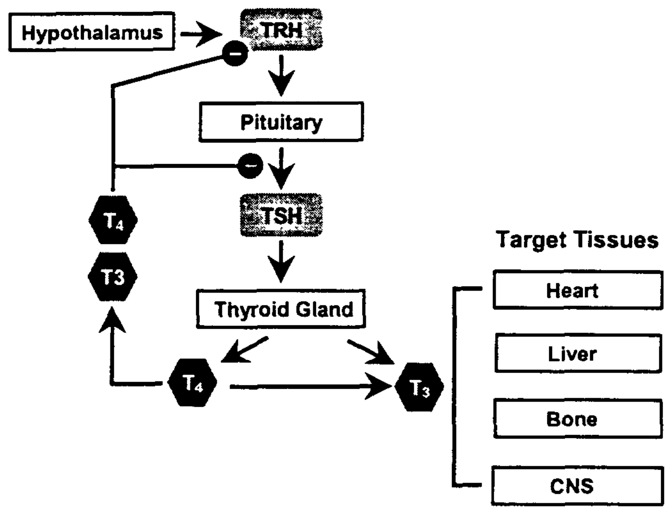

Figure 1. Basic Schematic of the Thyroid Hormone System

The pituitary is the key "biosensor" in the feedback loop, with the magnitude of TSH release controlled primarily by blood levels of T_4 and T_3 and some "fine-tuning"contributed by the TRH level. Figure 2 demonstrates the inverse relationship between TSH and free T_4 levels, as observed in over 500 ambulatory subjects.[3]

Linear regression analysis of the data points demonstrates that, for every 2-fold change in free T_4, the TSH level will change 100-fold (refer to triangle on the graph). Thus, TSH is considered to be the most sensitive measure of thyroid function, and is used clinically for the diagnosis and monitoring of thyroid patients. In fact, the diagnosis of hypo- or hyperthyroidism rests on the finding of an abnormal TSH, which is more sensitive than an abnormal T_4. Furthermore, titration of LT_4 dosage is performed by monitoring TSH levels, and a euthyroid state is considered achieved when TSH levels move to within the normal range (see Section 4.1).

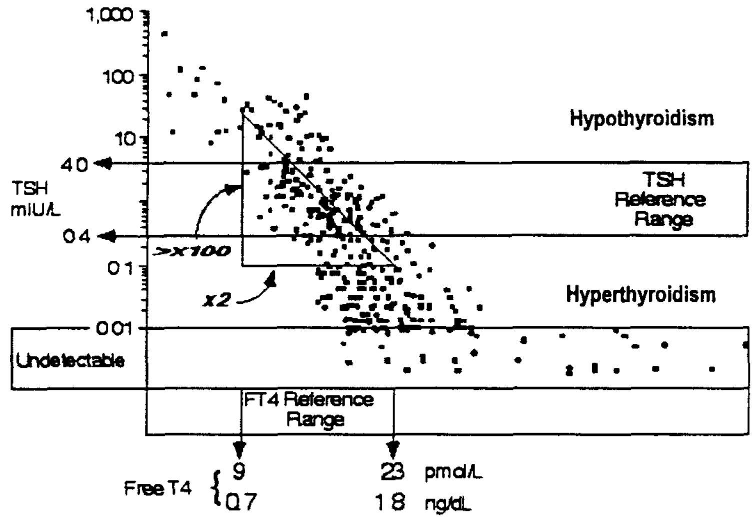

Figure 2. The Relationship Between Serum TSH And Free T_4 Concentrations in Individuals With Stable Thyroid Status and Normal Hypothalamic-Pituitary Function.

In summary, thyroid hormones are produced by the thyroid gland and regulated by a complex control system such that, in healthy subjects with normal thyroid function (euthyroid), T_4 and T_3 are tightly controlled within narrow ranges.

3.0 Assessment of the Current Guidance for LT_4 Bioequivalence

3.1 Background and rationale for M02-417 study

Evaluation of the pharmacokinetic curves generated for the NDA filings of LT_4 products led Abbott to question the sensitivity of bioavailability studies done in healthy volunteers with no adjustment made for the endogenous baseline concentrations of T_4. We hypothesized that given the magnitude of the endogenous T_4 measured at baseline, LT_4 products with large differences in bioavailability could be declared bioequivalent if this method were used. The current FDA bioequivalence methodology is to evaluate pharmacokinetic (PK) parameters using healthy volunteers, comparing 600 μg of the test compound to 600 μg of the reference compound in a crossover study, without correction for endogenous T_4 baseline level.[4] Abbott conducted a "bioequivalence" study in healthy volunteers using known dosages of a single formulation of LT_4 (Synthroid®) to test the sensitivity of the current FDA Guidance. We evaluated if the current methodology was able to differentiate two known lower dosages (400 and 450 µg) from the reference dose of 600 µg. We went on to evaluate the impact of various methods of correcting for endogenous T_4 baseline on the bioequivalence assessment in this study.

3.2 Results of M02-417 study

Results for bioequivalence assessment are presented below for 400 µg *versus* 600 µg, 450 µg *versus* 600µg, and 450 µg *versus* 400 µg, using PK parameters uncorrected for baseline T_4 levels and corrected for baseline T_4 levels are listed below.

3.2.1 T_4 without correcting for endogenous T_4 baseline concentrations

The relative bioavailabilities for the 450 µg and 400 µg doses as compared to the reference dose of 600 µg, using PK parameters (C_{max} and AUC_{48}) of T_4 without correction of the baseline are listed in Table 1. In addition, the relative bioavailability of 450 µg compared to the 400 µg is listed.

Table 1. Bioequivalence and Relative Bioavailability–Uncorrected Levothyroxine (T_4)

Regimens Test *vs.* Reference	Pharmacokinetic Parameter	Central Value*		Relative Bioavailability	
		Test	Reference	Point Estimate⁺	90% Confidence Interval
450 µg *vs.* 600 µg	C_{max}	13.0	14.0	0.928	0.890 – 0.968
	AUC_{48}	481.7	504.8	0.954	0.927 – 0.982
400 µg *vs.* 600 µg	C_{max}	12.9	14.0	0.921	0.883 – 0.960
	AUC_{48}	469.6	504.8	0.930	0.904 – 0.958
450 µg *vs.* 400 µg	C_{max}	13.0	12.9	1.007	0.967 – 1.050
	AUC_{48}	481.7	469.6	1.026	0.997 – 1.055

* Antilogarithm of the least squares means for logarithms.
\+ Antilogarithm of the difference (test minus reference) of the least squares means for logarithms.

Bioequivalence is concluded for each of the comparator pairs (450 µg *versus* 600 µg; 400 µg *versus* 600 µg and 450 µg *versus* 400 µg) because the 90% confidence intervals from the analyses of the natural logarithms of C_{max} and AUC_{48} are within the 0.80 to 1.25 range.

3.2.2 T_4 after correction for endogenous T_4 baseline concentrations

Three methods of correction were evaluated. These three methods are defined in Appendix A, Criteria for Evaluation. The relative bioavailabilities for the 450 µg and 400 µg doses as compared to the reference dose of 600 µg, using PK parameters (C_{max} and AUC_{48}) of T_4 with correction of the baseline (Correction Method 3) are listed in Table 2. The relative bioavailability of 450 µg compared to the 400 µg is also listed.

Results using Correction Method 3 are listed here because the point estimates for relative bioavailability as defined by AUC_{48} were generally further from unity than were the point estimates for that parameter using Corrections Method 1 and 2. The results using the other correction methods are listed in the expanded summary of the M02-417 study (see Appendix A for details). The determination for bioequivalence did not differ, no matter which correction method was used.

Table 2. Bioequivalence and Relative Bioavailability for T_4 (Correction Method 3)

Regimens Test *vs.* Reference	Pharmacokinetic Parameter	Central Value* Test	Central Value* Reference	Relative Bioavailability Point Estimate+	Relative Bioavailability 90% Confidence Interval
450 µg *vs.*600 µg	C_{max}	5.7	6.9	0.820	0.757 – 0.888
	AUC_{48}	125.1	172.9	0.723	0.672 – 0.779
400 µg *vs.* 600 µg	C_{max}	5.3	6.9	0.775	0.715 – 0.839
	AUC_{48}	115.4	172.9	0.667	0.620 – 0.718
450 µg *vs.* 400 µg	C_{max}	5.7	5.3	1.058	0.979 – 1.145
	AUC_{48}	125.1	115.4	1.084	1.008 – 1.165

* Antilogarithm of the least squares means for logarithms.
\+ Antilogarithm of the difference (test minus reference) of the least squares means for logarithms.

These analyses indicate that the use of baseline corrected T_4 pharmacokinetic parameters allow 400 and 450 µg to be differentiated from 600 µg. However, analyses using these simple methods of correction, each method limited by its inherent assumptions, failed to distinguish 450 µg from 400 µg.

3.2.3 Other observations

Analysis of the T_4 concentration data obtained during the 24 hours prior to the administration of the PK dose for each period confirmed that T_4 has a diurnal cycle.

Likewise, the serum concentrations of TSH showed a clear diurnal variation for Study Day –1 of each period. Administration of all three doses had homeostatic effects, but did not completely suppress the serum TSH concentration during the 24 hours following the PK dose. Analyses of the AUC_{24} for Study Day –1 revealed that the regimens (dose levels) had statistically significant different carryover effects from one period to the next (first-order carryover) and from Period 1 to Period 3 (second-order carryover).

3.3 Conclusions from M02-417 study

First, the results indicate that the use of baseline uncorrected T_4 pharmacokinetic parameters would result in declaring two products bioequivalent when they actually differ by as much as 25% to 33% (450 µg and 400 µg *versus* 600 µg). Considering the margin by which the conditions for declaring bioequivalence were passed in this study, products that differ by even more than 33% would also have a high likelihood of being declared bioequivalent products, stemming from the significant and complex contribution of endogenous T_4.

Second, the results from this study indicate that the use of baseline corrected T4 pharmacokinetic parameters would reduce the likelihood that two products would be declared bioequivalent when they actually differ by 25% to 33%. However, analyses using three simple methods of correction, each method limited by its inherent assumptions, failed to distinguish 450 µg from 400 µg. This is a 12.5% difference which, when applied to the range of doses typically used in clinical practice, is a clinically significant difference, as reflected in product labeling, clinical usage, and data from clinical studies.

Furthermore, it is apparent that simple methods of correction for endogenous T_4 concentrations in healthy volunteers are inadequate since these concentrations not only fluctuate on a diurnal cycle but may also be differentially affected by products with different rates and extents of absorption. Additionally, there is evidence of significant carryover from one dosing period to subsequent periods even with washout periods up to 53 days.

This study illustrates important flaws in the design and analysis of single-dose crossover studies in healthy volunteers to assess bioequivalence of LT_4 products, stemming from the significant and complex homeostatic mechanisms associated with administration of supraphysiologic doses of LT_4. We now know that better characterization of and correction for endogenous T_4 is required to provide proper interpretation of results in healthy volunteer studies. Alternative approaches to account for endogenous T_4 need to be identified and investigated. A change to the current FDA criterion beyond adding a simple correction for baseline T_4 is necessary.

4.0 LT_4 Therapy and the Clinical Consequences of Under- or Over-Treatment

Levothyroxine sodium is the treatment of choice as replacement or supplemental hormone therapy, or to suppress pituitary TSH in the treatment of thyroid carcinomas and nodules. According to the DOSAGE AND ADMINISTRATION section in the product labels for all levothyroxine sodium products, [5-7] "The goal of replacement therapy is to achieve and maintain a clinical and biochemical euthyroid state. The goal of suppressive therapy is to inhibit growth and/or function of abnormal thyroid tissues." The fundamental guiding principle of therapy is the maintenance of TSH in the desired range by individual titration of LT_4 dose.

4.1 TSH is the measurement of adequacy of treatment

Professional societies and product labels state that TSH is the biochemical endpoint to determine the thyroid hormone status. [5-10] The recommended management (under LABORATORY TESTS) is as follows: "The diagnosis of hypothyroidism is confirmed by measuring TSH levels using a sensitive assay....and measurement of free-T_4. The adequacy of therapy is determined by periodic assessment of appropriate laboratory tests and clinical evaluation."

The labels recommend testing in ADULTS, as follows: "The frequency of TSH monitoring during levothyroxine dose titration depends on the clinical situation but it is generally recommended at 6-8 week intervals until normalization. For patients who have recently initiated levothyroxine therapy and whose serum TSH has normalized or in patients who have had their dosage or brand of levothyroxine changed, the serum TSH concentration should be measured after 8-12 weeks. When optimum replacement dose has been attained, clinical (physical examination) and biochemical monitoring may be performed every 6-12 months, depending on the clinical situation, and whenever there is a change in the patient's status. It is recommended that a physical examination and a serum TSH measurement be performed at least annually…" [5-7]

The product labels and professional societies recognize the importance of using TSH measurements as the endpoint for evaluating the biochemical thyroid status. They state that once the patient is stabilized on an LT_4 dose, periodic assessment needs only be done every six to twelve months. [5-8, 10]

4.2 LT_4 therapy is individualized and carefully titrated

Treatment with LT_4 products is individualized for each patient, based on their underlying thyroid status, age, and presence or absence of other clinical conditions, particularly their cardiac function. With the exception of young healthy thyroid patients, the treated hypothyroid population is initiated with a low LT_4 dose and titration of the dose is done in small increments until they are able to achieve their euthyroid state. Thyroid cancer patients are carefully titrated to keep their TSH levels in the marginally hyperthyroid range. Recalling the inverse log-linear relationship of TSH to T_4 levels, titration to the marginally hyperthyroid state can require small dose increments.

In recognition of the narrow therapeutic window for serum T_4 and TSH, and the log-linear relationship between TSH and T_4 levels, professional societies and the product labels recommend that careful monitoring and titration be done when instituting LT_4 therapy. [5-8, 10] Because of the cardiac and cardiovascular consequences of rapid replacement or over-replacement, definitive recommendations are provided for special patient populations. Under DOSAGE AND ADMINISTRATION – SPECIAL PATIENT POPULATIONS the product label recommends "For most patients older than 50 years of age or for patients under 50 years of age with underlying cardiac disease, an initial starting dose of **25-50 mcg/day** of levothyroxine sodium is recommended, with gradual increments in dose at 6-8 week intervals, as needed. The recommended starting dose for elderly patients with cardiac disease is **12.5-25 mcg/day**, with gradual dose increments at

4-6 week intervals. The levothyroxine sodium dose is generally adjusted in 12.5-25 mcg increments until the patient with primary hypothyroidism is clinically euthyroid and the serum TSH has normalized." [5-7]

The medical literature on which FDA based its decision to approve oral levothyroxine tablets uniformly emphasizes the clinical need for fine dosing increments. As FDA stated in its review of Unithroid, "a 25 mcg dosage strength that meets chemistry and biopharm criteria for approval, is essential for proper labeling of the product for safe and effective use given that in certain clinical situations, levothyroxine sodium dosing is initiated at 12.5-25 mcg/day and increased in 12.5-25 mcg dosing increments." [11]

4.3 Fine-dosing increments – importance and medical use

The FDA recognizes that multiple dose strengths are required to accomplish adequate treatment of the thyroid patient population. The FDA, in a final agency decision regarding the regulatory status of Synthroid®, emphasized that PATIENTS NEED A PRECISE DOSE OF LEVOTHYROXINE SODIUM. "The dosage of replacement therapy is increased in gradual increments until the TSH test indicates the correct maintenance dosage has been achieved. In order to allow for fine adjustments of dose, which are necessary due to levothyroxine sodium's narrow therapeutic range, levothyroxine sodium products are marketed in an unusually large number of dosage strengths. Synthroid®, for example, comes in 25, 50, 75, 88, 100, 112, 125, 150, 175, 200, and 300 mcg strengths." [12]

Market research demonstrates that 1 in 5 dosage changes is an increase or decrease by 12 or 13 µg.The impact on thyroid hormone status of small deviations from an optimally titrated dose is demonstrated in a prospective, longitudinal study by Carr et al. Twenty-one patients on LT_4 replacement therapy for hypothyroidism were studied while taking the dose that produced a normal TSH response, and then the patients were restudied at lower and/or higher doses (Figure 3). [14] Dosage changes of as little as 25 µg rendered the patients either hypothyroid or hyperthyroid, dependent upon the direction of the dose change from the dose that maintained them in a euthyroid state.

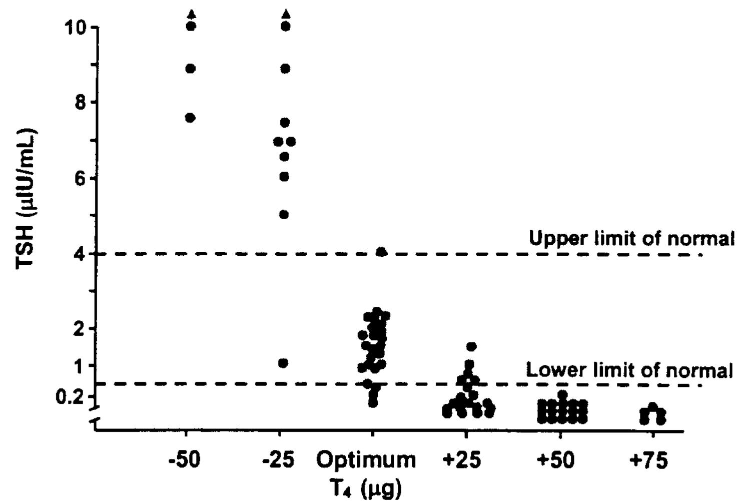

Figure 3. Resultant TSH levels With Incremental 25 µg Changes in LT_4 Dosage

Applying the inverse log-linear relationship of serum TSH to T_4 levels, these data would predict that dose changes that were half those studied in the Carr study would also render some, if not all, of the patients outside of the normal TSH range (0.4 to 4.0 mIU/L).

The ability to carefully titrate and maintain patients in the desired thyroid state is of paramount importance. The FDA acknowledged the same goals when approving the class labeling for all LT_4 products. In the product labels under PRECAUTIONS it states, "Levothyroxine has a narrow therapeutic index. Regardless of the indication for use, careful dosage titration is necessary to avoid the consequences of over- or under-treatment. These consequences include, among others, effects on growth and development, cardiovascular function, bone metabolism, reproductive function, cognitive function, emotional state, gastrointestinal function, and on glucose and lipid metabolism."

4.4 Clinical consequences of hypothyroidism and hyperthyroidism

4.4.1 Patient populations treated with LT_4 products

Functional thyroid disease can manifest as either over- or under-active thyroid hormone status. [15, 16] In either case, there is a wide spectrum of the clinical expression of the disease from mild to severe. However, the clinical consequences of each are more severe the further the thyroid function has deviated from normal, i.e., severe thyrotoxicosis (severe hyperthyroidism) and myxedema coma (severe hypothyroidism). Significant clinical consequences also occur with milder forms of the disease ("subclinical" thyroid disease) as may be seen when the patient is not treated to reach and maintain the euthyroid state. In a large health screening study of 25,862 subjects in Colorado, 18% of all patients treated with LT_4 products had TSH levels above the upper limit of the normal range, indicating that those patients were in a subclinical hypothyroid state despite LT_4 treatment. [17]

The American Cancer Society projects the number of new cases of thyroid cancer in 2003 will reach 22,000 with an annual mortality of 1,400. [18] The low mortality rates for this cancer is due in part to the effectivness care delivered for these patients. Thyroid cancer patients undergo surgical removal of their thyroid gland and treatment with radioactive iodine to ablate the remaining thyroid cancer cells. Thereafter, they are purposefully maintained in a marginally hyperthyroid state (TSH < 0.4 mIU/L). [5-7, 9, 19] TSH is a growth factor for normal and cancerous thyroid cells. The goal of LT_4 treatment is to deliver adequate LT_4 to suppress the TSH to just below the normal range. Lowering TSH levels removes the growth stimulus, thereby reducing the probability that any remaining thyroid cancer cells will grow to be of any clinical significance. If these patients are under-treated, they are at risk of having a recurrence of their thyroid cancer or development of metastases. Conversely, if they receive too much LT_4 they are at risk of the complications of over-treatment, described below.

4.4.2 Consequences of hypothyroidism and hyperthyroidism

It is paramount that patients be guaranteed that any LT_4 product substitution produce the same therapeutic response such that the efficacy and safety profile they rely upon is not compromised.

The FDA, in a final agency decision regarding the regulatory status of Synthroid, described the safety risks when patients are inadvertently over- or under-treated. "Superpotent tablets of levothyroxine sodium pose safety risks. Patients who inadvertently receive more levothyroxine than is necessary to control their condition may experience angina, tachycardia, or arrhythmias. There is also evidence that overtreatment can contribute to osteoporosis. Subpotent tablets of levothyroxine sodium are not adequately effective and, therefore, also pose safety risks. Patients inadvertently receiving less than their proper dose may experience such symptoms as fatigue, lethargy, sleepiness, mental impairment, depression, cold intolerance, hair loss, hoarseness, weight gain, constipation, decreased appetite, dry skin, increased perspiration, arthralgia, menstrual disturbances, and paresthesias. Because of the serious consequences of too much or too little circulating thyroxine, it is very important that patients receive the dose of levothyroxine sodium determined by their physicians to be optimal to replace the amount of hormone that would have been present naturally." [12]

The FDA stated that the potential side effects that occur with mild hypothyroidism and hyperthyroidism involve many different organ systems. [12] Some specific examples highlight the importance of maintaining thyroid hormones in their narrow therapeutic ranges. Maternal thyroid hormone status, particularly during early pregnancy, is important to the well being of the pregnant woman's offspring. Early in pregnancy the fetus is totally dependent on receiving thyroid hormone from the mother. [20] Hypothyroidism during pregnancy has been associated with lower IQ scores in the children. [21]

Mild thyroid failure is associated with elevated total cholesterol and LDL-cholesterol levels. [17, 22-26] This is consistent with the finding that thyroid hormone is a positive regulator for the production of LDL-receptors. [2] In the hypothyroid state removal of LDL-cholesterol particles from the plasma into the liver and other tissues would be limited. Hypothyroidism is an independent risk factor for myocardial infarction. [27]

Hypothyroidism is associated with a slow heart rate (bradycardia) and decreased contractility of heart muscle. [28] Thyroid hormone-responsive genes have been identified that are consistent with these clinical findings. [1, 28] Clinical practice guidelines and product labels for LT_4 products advise careful monitoring and treatment of thyroid disease patients who also suffer from heart failure, as both hypo- and hyperthyroidism can worsen the heart failure. [5-8, 10] As testing of cardiac function becomes more sophisticated, it is evident that even mild thyroid failure has a significant effect on cardiac muscle contractility. [29-31] In the hypothyroid patient, with each heartbeat, less blood is pumped from the heart to the rest of the body and increased backward pressure causes fluid to build up in the lungs and legs. Hyperthyroidism is associated with rapid heartbeat (tachycardia) and atrial fibrillation. [28] Both of these also result in less blood being pumped to the rest of the body with each heartbeat. Atrial fibrillation is also associated with an increased risk of stroke. [32]

4.5 Summary of LT_4 therapy and clinical consequences

In summary, physicians use the TSH levels to judge the adequacy of treatment. To achieve treatment goals, physicians and the 13 million LT_4 treated patients in the U.S. rely on multiple dosage strengths. For titration and maintenance of the desired thyroid status, dosage strengths that differ by only 12 to13 µg are frequently used. Changes in LT_4 treatment or potency, with resulting changes in TSH levels, have significant clinical consequences.

The concerns of the FDA as outlined above and recommendations in the LT_4 product labels further emphasize the concern that LT_4 product substitution should only be done when LT_4 can be delivered so as not to produce over- or under-treatment. This takes on particular significance for patients who have their TSH monitored every 6 to 12 months. It is possible that LT_4 product substitution could occur between two consecutive TSH assessments and the patient's thyroid status could be changed toward under- or over-treatment. It is these patients who have an increased health risk from a product that is supra- or sub-potent.

5.0 Conclusions

The determination of bioequivalence of LT_4 products should signify that, under all circumstances, these products are truly interchangeable without adverse clinical consequences, and without the need for clinical monitoring, retesting and retitration. The goal of thyroid hormone replacement therapy for hypothyroid patients is to safety titrate the patient to the appropriate dose that achieves and then maintains the euthyroid state. The goal of TSH suppression for the treatment of thyroid cancer is to remove the growth-promoting effect of TSH on thyroid cancer cells such that the patient does not suffer regrowth of the cancer. For these clinical purposes, patients and their physicians rely upon serum TSH levels, the most sensitive and easily measurable parameter of thyroid hormone function. To achieve the optimal TSH levels, physicians titrate individual patients using a wide range dosage forms, routinely using dosage increments in the 12-13 μg range. The clinical evidence presented here demonstrates that small changes in T_4 dosage result in TSH levels that are correlated with undesirable clinical consequences.

The current FDA guidance for the assessment of LT4 bioequivalence does not account for endogenous thyroxine or its biologic regulation. Results from the "bioequivalence" study (M02-417) reveal that LT_4 products approved using the current FDA criterion and based on bioequivalence data without baseline correction for endogenous T_4 levels could differ by as much as 33% from the reference product. Furthermore, simple corrections of the T_4 baseline did not sufficiently solve the problem, because two products that differed by 12.5% in thyroxine content would be declared bioequivalent. Other methodological flaws were also observed that could further reduce the reliability of the current guidance to ensure that products declared bioequivalent will be substitutable in patients without adverse clinical consequences, and without the need to remonitor, retest and retitrate.

In summary, we recommend that the new data be taken into account and careful consideration be given to developing a specific guidance for the assessment of bioequivalence of levothyroxine sodium products. To do so, this guidance must adequately consider the unique nature of the thyroid hormone system and the demonstrated limitations of the current criteria even with baseline correction. Physicians and patients rely on dosage strengths that differ by only 12-13 μg. The concerns of the FDA as outlined in the FDA final action for Synthroid® and recommendations in the LT_4 product labels further emphasize the concern that LT_4 product substitution should only be done when LT_4 can be delivered so as not to produce over- or under-treatment. This needs to be ensured because physicians and patients alike rely on receiving "the correct dose when filling and refilling their carefully calculated prescriptions." [12] The necessity to deliver bioequivalent LT_4 products assumes that the methodology used to determine bioequivalence is robust and sensitive enough to differentiate doses of LT_4 that are truly different.

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Appendix A

Abbott Laboratories
Study M02-417 Synopsis and Discussion

Title of Study

Evaluating the Impact of Correcting for Endogenous T_4 Baseline on the Bioequivalence of Levothyroxine Sodium Formulations in Healthy Volunteers

Objective

The objective of this study was to evaluate the impact of various methods for correcting for endogenous T_4 baseline on the bioequivalence of levothyroxine sodium formulations in healthy volunteers.

Methodology

This Phase 1, single-dose, open-label, study was conducted according to a three-period, randomized crossover design in healthy volunteers. The total dose given was 600 µg levothyroxine sodium for Regimen A, 450 µg levothyroxine sodium for Regimen B and 400 µg levothyroxine sodium for Regimen C. Subjects received one of six sequences of Regimen A (twelve 50 µg Synthroid® tablets), Regimen B (nine 50 µg Synthroid® tablets) or Regimen C (eight 50 µg Synthroid® tablets) under fasting conditions at approximately 0830 on Study Day 1 of each period. A washout interval of at least 44 days separated the doses of the three study periods.

Blood samples (sufficient to provide approximately 2 mL serum) for total levothyroxine (T_4), total triiodothyronine (T_3) and thyroid stimulating hormone (TSH) assay were collected by venipuncture into 5 mL evacuated siliconized collection tubes as follows:

- At approximately 0 hours and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 18 hours after the 0-hour collection on Study Day –1 in each study period.
- At approximately –30 minutes, –15 minutes and at 0 hours prior to dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48, 72 and 96 hours after dosing on Study Day 1 in each study period.

Serum concentrations of T_4 and T_3 were determined using validated radioimmunoassay (RIA) methods. The lower limit of quantification of T_4 was 1.00 µg/dL. The lower limit of quantification of T_3 was 0.25 ng/mL. Serum concentrations of TSH were determined using a validated IRMA assay; lower limit of quantification was 0.250 µIU/mL.

Subjects

Subjects were male and female volunteers between 19 and 50 years of age, inclusive. Subjects were judged to be euthyroid and in general good health based on the results of medical history, physical examination, vital signs, 12-lead electrocardiogram and laboratory tests. Females were postmenopausal, sterile, or if of childbearing potential, were not pregnant or breast-feeding and were practicing an acceptable method of birth control.

Thirty-six subjects (18 M, 18 F) participated in the study, with mean age of 32.9 years, mean weight of 74.5 kg and mean height of 172 cm. Three subjects received study drug in only one period and thus were not included in any of the pharmacokinetics analyses. Thirty-three subjects (16 M, 17 F) were included in the pharmacokinetic analyses, with mean age of 33.1 years, mean weight of 73.5 kg and mean height of 171 cm.

Pharmacokinetics and Statistical Methods

The pharmacokinetic parameters of total levothyroxine (T_4) were estimated using noncompartmental methods. These included: the maximum serum concentration (C_{max}) and time to C_{max} (T_{max}), the area under the serum concentration-time curve (AUC) from time 0 to 48 hours (AUC_{48}), time 0 to 72 hours (AUC_{72}) and time 0 to 96 hours (AUC_{96}). For T_4, values of these parameters (C_{max}, T_{max}, AUC_{48}, AUC_{72} and AUC_{96}) were determined without correction for endogenous T_4 levels and after correcting all post-dose concentrations using each of following three methods:

Correction Method 1: The predose baseline value on the day of dosing was subtracted from each post-dose concentration. The pre-dose baseline value was calculated as the average of the three concentrations at –0.5, –0.25 and 0 hours prior to dosing in each period.

Correction Method 2: For each time of post-dose sampling, the observed concentration was corrected assuming that the endogenous T_4 baseline level at 0 hours declines according to a half-life of 7 days.

Correction Method 3: The T_4 concentration for each time of post-dose sampling was corrected by the concentration observed at the same time of day during the 24 hours preceding the dose.

For all three methods of correction, the corrected 0-hour concentration was assumed to be 0.

For uncorrected and corrected T_4 an analysis of variance (ANOVA) with fixed effects for sex, sequence, sex-by-sequence interaction, period, regimen and the interaction of sex with each of period and regimen, and with random effects for subjects nested within sex-by-sequence combination was performed for T_{max}, and the natural logarithms of C_{max} AUC_{48}, AUC_{72} and AUC_{96}. A significance level of 0.05 was used for all tests.

The bioavailability of each of Regimen B (450 µg dose) and Regimen C (400 µg dose) relative to that of Regimen A (600 µg dose) for uncorrected and corrected T_4 was assessed by the two one-sided tests procedure[1] *via* 90% confidence intervals obtained from the analysis of the natural logarithms of AUC_{48} and C_{max}. Bioequivalence was concluded if the 90% confidence intervals from the analyses of the natural logarithms of AUC_{48} and C_{max} were within the 0.80 to 1.25 range. Likewise, the bioavailability of Regimen B (450 µg dose) relative to that of Regimen C (400 µg dose) was assessed. The same was done using each of AUC_{72} and AUC_{96} in place of AUC_{48}.

A repeated measures analysis was performed on the T_4 concentration data of Study Day –1 for each period. To investigate the possibility of carryover effects, an ANOVA was performed on the logarithms of the Study Day –1 AUC_{24}.

Pharmacokinetic Results

Levothyroxine (T_4) Without Correcting for Endogenous T_4 Baseline Concentrations

The mean serum concentration-time plots for uncorrected T_4 after administration of levothyroxine sodium on Study Day 1 are presented in Figure 1. The mean T_4 serum concentrations-time profiles are fairly consistent after administration of the three regimens. Mean T_4 concentrations prior to dosing are approximately 7.5 µg/dL and increase to about 13 to 14 µg/dL at maximum before declining. The mean T_4 concentrations remain at approximately 9 µg/dL at 96 hours after administration of these large doses of levothyroxine sodium to the healthy volunteers.

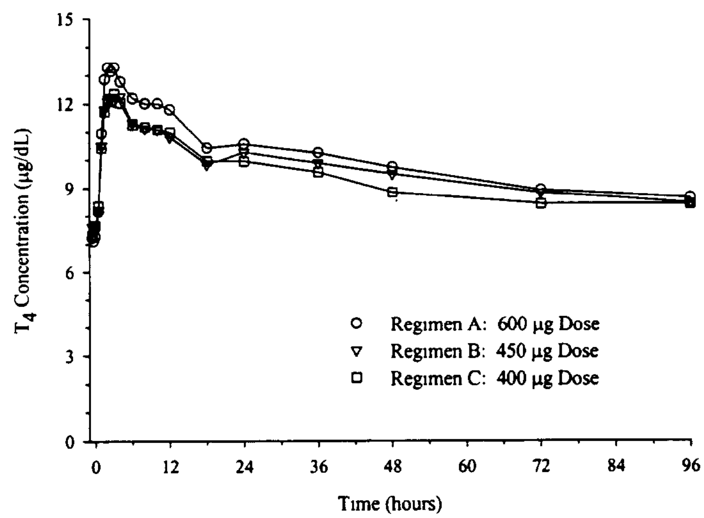

Figure 1. Mean Levothyroxine (T_4) Concentration-Time Profiles on Study Day 1 Following Single Dose Administration of Levothyroxine Sodium – Uncorrected for Endogenous T_4 Baseline Concentrations

Mean ± standard deviation (SD) pharmacokinetic parameters of T_4 after administration of the three regimens without correcting for endogenous T_4 baseline concentrations are listed in Table 1.

Table 1. Mean ± SD Pharmacokinetic Parameters of Levothyroxine (T_4) Without Correcting for Endogenous T_4 Baseline Concentrations

Pharmacokinetic Parameters (units)		Regimens A: 600 μg Dose (N = 31)	B: 450 μg Dose (N = 33)	C: 400 μg Dose (N = 33)
T_{max}	(h)	3.1 ± 2.4	3.2 ± 2.1	3.5 ± 3.3
C_{max}	(μg/dL)	14.3 ± 2.14	13.2 ± 2.05*	13.2 ± 2.45*
AUC_{48}	(μg•h/dL)	518 ± 71.8	493 ± 72.7*	484 ± 73.6*
AUC_{72}	(μg•h/dL)	741 ± 102	712 ± 108*	691 ± 102*,+
AUC_{96}	(μg•h/dL)	951 ± 133	919 ± 139	892 ± 133*,+

* Statistically significantly different from Regimen A (ANOVA, $p < 0.05$).
\+ Statistically significantly different from Regimen B (ANOVA, $p < 0.05$).

The bioequivalence/bioavailability results for uncorrected T_4 are listed in Table 2.

Table 2. Bioequivalence and Relative Bioavailability–Uncorrected Levothyroxine (T_4)

Regimens Test *vs.* Reference	Pharmacokinetic Parameter	Central Value*		Relative Bioavailability	
		Test	Reference	Point Estimate+	90% Confidence Interval
450 µg *vs.* 600 µg	C_{max}	13.0	14.0	0.928	0.890 – 0.968
	AUC_{48}	481.7	504.8	0.954	0.927 – 0.982
	AUC_{72}	694.9	721.9	0.963	0.936 – 0.990
	AUC_{96}	896.2	925.6	0.968	0.941 – 0.996
400 µg *vs.* 600 µg	C_{max}	12.9	14.0	0.921	0.883 – 0.960
	AUC_{48}	469.6	504.8	0.930	0.904 – 0.958
	AUC_{72}	670.4	721.9	0.929	0.903 – 0.955
	AUC_{96}	865.7	925.6	0.935	0.909 – 0.962
450 µg *vs.* 400 µg	C_{max}	13.0	12.9	1.007	0.967 – 1.050
	AUC_{48}	481.7	469.6	1.026	0.997 – 1.055
	AUC_{72}	694.9	670.4	1.037	1.009 – 1.065
	AUC_{96}	896.2	865.7	1.035	1.007 – 1.064

* Antilogarithm of the least squares means for logarithms.
\+ Antilogarithm of the difference (test minus reference) of the least squares means for logarithms.

Levothyroxine (T_4) After Correction for Endogenous T_4 Baseline Concentrations

The mean serum concentration-time plots for T_4, after correction for endogenous baseline levels of levothyroxine using each of the correction methods, are presented in Figure 2 for Correction Method 1, Figure 3 for Correction Method 2, and Figure 4 for Correction Method 3. The mean T_4 serum concentrations after correcting for endogenous baseline levels by any of the three methods of correction were higher after administration of Regimen A (600 µg dose) than after administration of Regimens B (450 µg dose) and C (400 µg dose) throughout the 96-hour sampling period. The mean baseline corrected T_4 concentrations for Regimens B (450 µg dose) and C (400 µg dose) were comparable throughout the 96-hour sampling period. The baseline corrected T_4 concentrations prior to dosing were assigned a value of zero for each of the three methods of correction. However, 96 hours after administration of these large doses of levothyroxine sodium to healthy volunteers the mean baseline corrected T_4 concentrations remain at approximately 1 to 2 µg/dL for Correction Methods 1 and 3 and approximately 3 to 4 µg/dL for Correction Method 2.

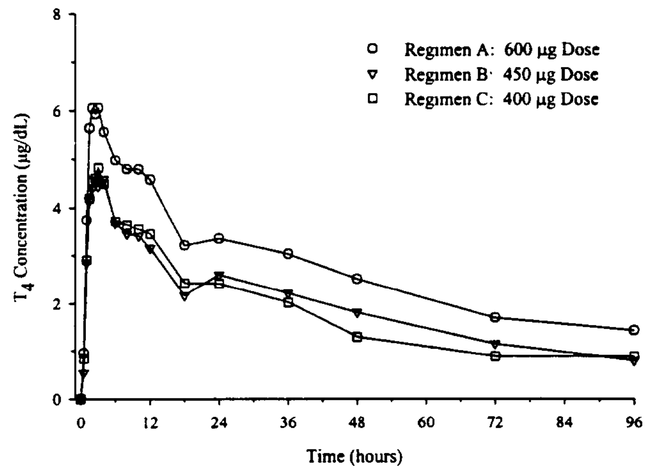

Figure 2. Mean Levothyroxine (T_4) Concentration-Time Profiles after Correction for Endogenous Baseline Levels of T_4 Using Correction Method 1

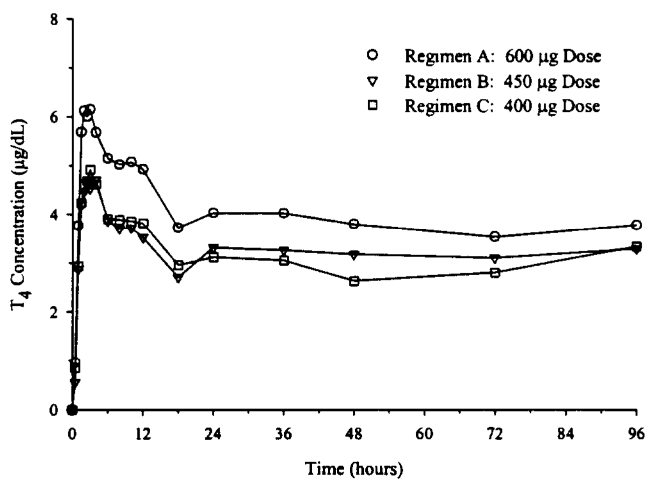

Figure 3. Mean Levothyroxine (T_4) Concentration-Time Profiles after Correction for Endogenous Baseline Levels of T_4 Using Correction Method 2

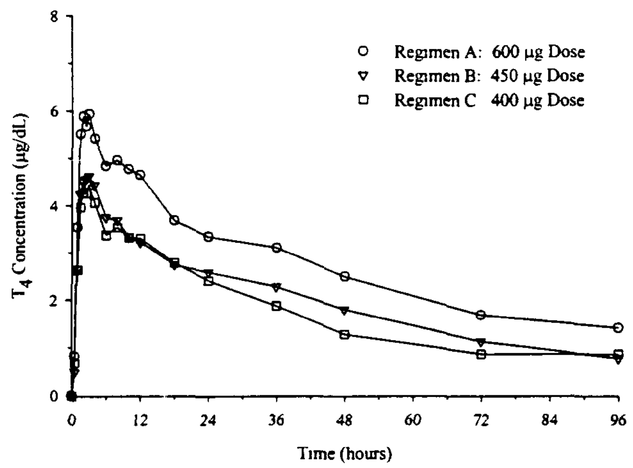

Figure 4. Mean Levothyroxine (T_4) Concentration-Time Profiles after Correction for Endogenous Baseline Levels of T_4 Using Correction Method 3

Mean ± SD pharmacokinetic parameters of T_4 after administration of the three regimens after correcting for endogenous T_4 baseline concentrations are listed in Table 3.

Table 3. Mean ± SD Pharmacokinetic Parameters of Levothyroxine (T_4) after Correcting for Endogenous T_4 Baseline Concentrations

Pharmacokinetic Parameters (units)		Regimens A: 600 µg Dose (N = 31)	B: 450 µg Dose (N = 33)	C: 400 µg Dose (N = 33)
Correction Method 1				
T_{max}	(h)	3.1 ± 2.4	3.2 ± 2.1	3.5 ± 3.3
C_{max}	(µg/dL)	7.05 ± 1.66	5.54 ± 1.53*	5.72 ± 1.44*
AUC_{48}	(µg•h/dL)	172 ± 40.4	126 ± 39.0*	123 ± 45.4*
AUC_{72}	(µg•h/dL)	222 ± 56.0	161 ± 55.5*	149 ± 68.6*
AUC_{96}	(µg•h/dL)	259 ± 72.5	184 ± 69.9*	169 ± 92.5*
Correction Method 2				
T_{max}	(h)	3.3 ± 2.8	5.8 ± 9.3	3.7 ± 3.5
C_{max}	(µg/dL)	7.15 ± 1.64	5.68 ± 1.50*	5.83 ± 1.45*
AUC_{48}	(µg•h/dL)	204 ± 40.9	160 ± 40.1*	156 ± 43.4*
AUC_{72}	(µg•h/dL)	292 ± 56.9	235 ± 58.2*	221 ± 62.7*
AUC_{96}	(µg•h/dL)	379 ± 74.0	312 ± 74.6*	295 ± 82.2*
Correction Method 3				
T_{max}	(h)	3.5 ± 3.1	3.6 ± 2.3	3.6 ± 4.0
C_{max}	(µg/dL)	7.03 ± 1.64	5.85 ± 1.78*	5.56 ± 1.69*
AUC_{48}	(µg•h/dL)	176 ± 36.9	131 ± 39.2*	120 ± 28.4*
AUC_{72}	(µg•h/dL)	226 ± 49.4	166 ± 52.9*	146 ± 45.4*,+
AUC_{96}	(µg•h/dL)	263 ± 64.8	189 ± 65.6*	167 ± 67.2*

* Statistically significantly different from Regimen A (ANOVA, $p < 0.05$).
\+ Statistically significantly different from Regimen B (ANOVA, $p < 0.05$).

The bioequivalence/bioavailability results for T_4 using Correction Method 1, Correction Method 2, and Correction Method 3 are listed in Tables 4, 5, and 6, respectively.

Table 4. Bioequivalence and Relative Bioavailability for T_4 (Correction Method 1)

Regimens Test *vs.* Reference	Pharmacokinetic Parameter	Central Value* Test	Central Value* Reference	Relative Bioavailability Point Estimate+	Relative Bioavailability 90% Confidence Interval
450 µg *vs.*600 µg	C_{max}	5.4	6.9	0.783	0.727 – 0.844
	AUC_{48}	119.7	167.3	0.715	0.658 – 0.778
	AUC_{72}	151.4	215.7	0.702	0.636 – 0.774
	AUC_{96}	170.2	250.2	0.680	0.602 – 0.768
400 µg *vs.* 600 µg	C_{max}	5.6	6.9	0.803	0.745 – 0.865
	AUC_{48}	118.9	167.3	0.711	0.653 – 0.773
	AUC_{72}	144.9	215.7	0.672	0.609 – 0.741
	AUC_{96}	165.1	250.2	0.660	0.584 – 0.746
450 µg *vs.* 400 µg	C_{max}	5.4	5.6	0.975	0.906 – 1.049
	AUC_{48}	119.7	118.9	1.007	0.926 – 1.094
	AUC_{72}	151.4	144.9	1.044	0.948 – 1.150
	AUC_{96}	170.2	165.1	1.031	0.914 – 1.163

* Antilogarithm of the least squares means for logarithms.
\+ Antilogarithm of the difference (test minus reference) of the least squares means for logarithms.

Table 5. Bioequivalence and Relative Bioavailability for T_4 (Correction Method 2)

Regimens Test *vs.* Reference	Pharmacokinetic Parameter	Central Value* Test	Central Value* Reference	Relative Bioavailability Point Estimate+	Relative Bioavailability 90% Confidence Interval
450 µg *vs.*600 µg	C_{max}	5.6	7.0	0.793	0.739 – 0.850
	AUC_{48}	154.5	199.1	0.776	0.721 – 0.835
	AUC_{72}	227.5	284.9	0.799	0.729 – 0.875
	AUC_{96}	301.6	369.5	0.816	0.743 – 0.897
400 µg *vs.* 600 µg	C_{max}	5.7	7.0	0.807	0.753 – 0.866
	AUC_{48}	148.4	199.1	0.745	0.693 – 0.802
	AUC_{72}	207.9	284.9	0.730	0.666 – 0.800
	AUC_{96}	277.3	369.5	0.750	0.683 – 0.824
450 µg *vs.* 400 µg	C_{max}	5.6	5.7	0.982	0.916 – 1.051
	AUC_{48}	154.5	148.4	1.041	0.969 – 1.119
	AUC_{72}	227.5	207.9	1.094	1.001 – 1.197
	AUC_{96}	301.6	277.3	1.088	0.992 – 1.192

* Antilogarithm of the least squares means for logarithms.
\+ Antilogarithm of the difference (test minus reference) of the least squares means for logarithms.

Table 6. Bioequivalence and Relative Bioavailability for T_4 (Correction Method 3)

Regimens Test *vs.* Reference	Pharmacokinetic Parameter	Central Value*		Relative Bioavailability	
		Test	Reference	Point Estimate+	90% Confidence Interval
450 µg *vs.* 600 µg	C_{max}	5.7	6.9	0.820	0.757 – 0.888
	AUC_{48}	125.1	172.9	0.723	0.672 – 0.779
	AUC_{72}	158.7	222.0	0.715	0.645 – 0.792
	AUC_{96}	177.7	256.6	0.693	0.631 – 0.760
400 µg *vs.* 600 µg	C_{max}	5.3	6.9	0.775	0.715 – 0.839
	AUC_{48}	115.4	172.9	0.667	0.620 – 0.718
	AUC_{72}	135.9	222.0	0.612	0.553 – 0.678
	AUC_{96}	164.0	256.6	0.639	0.582 – 0.702
450 µg *vs.* 400 µg	C_{max}	5.7	5.3	1.058	0.979 – 1.145
	AUC_{48}	125.1	115.4	1.084	1.008 – 1.165
	AUC_{72}	158.9	135.9	1.168	1.057 – 1.291
	AUC_{96}	177.7	164.0	1.084	0.989 – 1.188

* Antilogarithm of the least squares means for logarithms.
\+ Antilogarithm of the difference (test minus reference) of the least squares means for logarithms.

Baseline Levothyroxine (T_4) Prior to Dosing (Study Day –1)

The mean serum concentration-time plots for baseline T_4 on Study Day –1 prior to dosing with levothyroxine sodium in each Period are presented in Figure 5. Analysis of the T_4 concentration data obtained during the 24 hours of Study Day –1 of each period confirmed that T_4 has a diurnal cycle with statistically significant differences across time. The diurnal variation in baseline T_4 concentrations prior to dosing are consistent with the observed diurnal variation in the serum concentrations of TSH (Figure 6).

Analysis of the 24-hour AUC for Study Day –1 revealed that the regimens (dose levels) had statistically significantly different carryover effects from one period to the next (first-order carryover) and from Period 1 to Period 3 (second-order carryover).

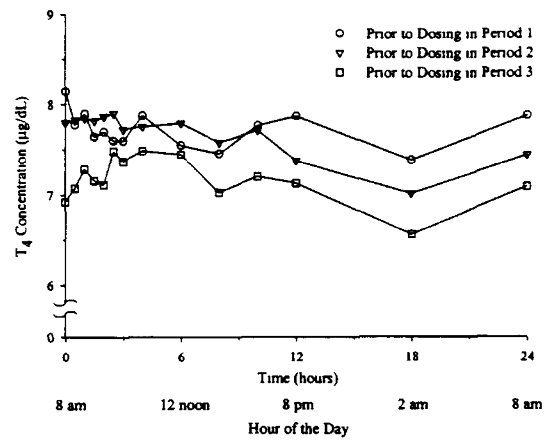

Figure 5. **Mean Levothyroxine (T_4) Concentration-Time Profiles on Study Day –1 Prior to Dosing with Levothyroxine Sodium by Period**

Thyroid-Stimulating Hormone (TSH)

The mean serum concentration-time plots for TSH for the 24 hours prior to and 96 hours after administration of levothyroxine sodium on Study Day 1 are presented in Figure 6. The serum concentrations of TSH appear to clearly show diurnal variation, prior to dosing. During the 24-hour period prior to dosing, the concentrations of TSH decline during the morning hours until reaching the lowest levels at approximately 1200 before starting to increase to maximum values at 0200 the next morning, *i.e.*, the morning of Study Day 1 (18 hour sample on Study Day –1).

Administration of any of the three large doses of levothyroxine sodium substantially, but not completely, suppressed the TSH serum concentrations throughout the 24-hour period after dosing on Study Day 1. TSH serum concentrations continued to be suppressed throughout the 96-hour sampling period after dosing; the concentrations did not return to baseline values even after 96 hours. The rank order of suppression of the TSH serum concentrations was consistent with the rank order of the size of levothyroxine sodium dose administered in each of the three regimens with the greatest suppression of TSH serum concentrations associated with administration of the largest dose (Regimen A, 600 µg).

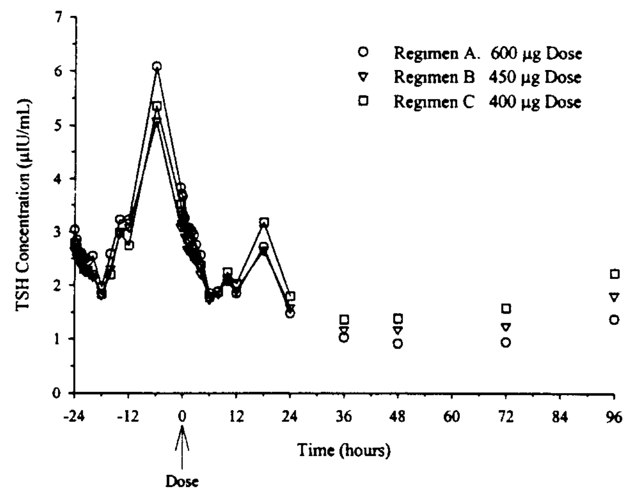

Figure 6. Mean TSH Concentration-Time Profiles for the 24 Hours Prior to (Study Day –1) and for the 96 Hours after Administration of Levothyroxine Sodium on Study Day 1

Triiodothyronine (T_3) Concentrations

The mean T_3 concentration for the 24-hour period prior to dosing and throughout the 96-hour period after dosing were in the very narrow range of 1.1 to 1.3 ng/mL after administration of the large doses of levothyroxine sodium to healthy volunteers.

Discussion

Determination of the bioavailability of levothyroxine sodium products in healthy volunteers presents significant challenging issues. Levothyroxine is naturally present in the blood, with total endogenous baseline T_4 levels ranging from 4 to 14 µg/dL. Thus, to compare the bioavailabilities of levothyroxine sodium formulations after a single dose in healthy volunteers, FDA Guidance[2] recommends administration of 600 µg, several times the normal clinical dose, to raise the levels of the drug significantly above baseline and to hopefully reduce the influence of endogenous levels. However, results from several bioavailability studies and a stochastic simulation study with levothyroxine products suggested that, given very reasonable assumptions about endogenous levothyroxine behavior in healthy subjects, the use of baseline uncorrected C_{max} and AUC_{48} values would result in a high probability of declaring two products bioequivalent when they actually differ by as much as 35%.[3]

The current study was designed to evaluate how much two formulations could differ and still pass the bioequivalence criteria specified in the current guidance when not correcting for endogenous T_4 baseline levels. The results from this study clearly indicate that the use of baseline uncorrected C_{max}, AUC_{48}, AUC_{72} and AUC_{96} values would result in declaring two products bioequivalent when they actually differ by as much as 25% to 33% (450 µg and 400 µg *versus* 600 µg). Utilizing the criteria specified in FDA Guidance,[2] both the 450 µg dose (Regimen B) and the 400 µg dose (Regimen C) would be declared bioequivalent to the 600 µg dose (Regimen A) because the 90% confidence intervals for evaluating bioequivalence obtained without correcting for endogenous T_4 baseline levels were contained within the 0.80 to 1.25 range. Furthermore, the 450 µg dose would be declared bioequivalent to the 400 µg dose because the 90% confidence intervals for evaluating bioequivalence without correcting for endogenous T_4 baseline levels were contained within the 0.80 to 1.25 range. Considering the margin by which the conditions for declaring bioequivalence were passed in this study, products that differ by more than 33% would have a good chance of being declared bioequivalent on the basis of uncorrected data. The results of this study clearly demonstrate the significant limitations and problems with the current methodology and criteria for assessing the bioequivalence of levothyroxine sodium products in healthy volunteers without correcting for endogenous T_4 baseline levels.

Several mathematical and statistical methods can be used to correct for the contribution of T_4 baseline levels, based on different biologic assumptions about the behavior of endogenous T_4 following administration of exogenous levothyroxine. When a single dose of exogenous levothyroxine sodium is given to healthy subjects, one could assume that endogenous levothyroxine levels remain constant if there is no suppression of endogenous production (Correction Method 1). If production were completely suppressed, *via* feedback through the hypothalamic-pituitary axis, the endogenous levothyroxine would decline at an average rate defined by its half-life, which is approximately 7 days (Correction Method 2). Thus, a constant baseline of endogenous levothyroxine (Correction Method 1) *versus* a baseline that decays exponentially with a 7-day half-life (Correction Method 2) defines the limits for endogenous levothyroxine following a dose of exogenous levothyroxine sodium. This assumes that no other components of the thyroid system would impact the turnover of T_4 and T_3. The third method of baseline correction (Correction Method 3) employed in this study corrected the T_4 concentration for each time of post-dose sampling by the baseline T_4 concentration observed at the same time of day during the 24 hours preceding the dose, *i.e.*, on Study Day –1.

One of the objectives of the current study was to better understand the impact of three different methods of correction for endogenous T_4 baseline on the bioequivalence evaluation of levothyroxine sodium formulations in healthy volunteers. In contrast to the results with uncorrected data, for all three correction methods for endogenous T_4 baseline, neither the 450 µg dose nor the 400 µg dose would be declared bioequivalent to the 600 µg dose. However, as with the uncorrected data, the 450 µg dose would continue to be declared bioequivalent to the 400 µg dose after correcting for endogenous T_4 baseline levels using any of the three correction methods because the 90% confidence intervals for evaluating bioequivalence after correcting for endogenous T_4 baseline continue to be contained within the 0.80 to 1.25 range. The 50µg difference between the 450 µg dose and the 400 µg dose represents a 12.5% difference.

Correction Method 1 relies on the assumption that there is no suppression of endogenous production when a single large dose of exogenous levothyroxine sodium is given to healthy subjects, thus assuming a constant baseline of endogenous levothyroxine. This assumption is clearly not true since TSH levels after dosing with levothyroxine sodium in the study were definitely suppressed, though not completely. Thus, it is very unlikely that endogenous T_4 production would be constant after administration of large doses of levothyroxine sodium to healthy volunteers. This method of correction has also several undesirable characteristics. The method will sometimes produce a negative value for AUC as was observed with one of the subjects in this study. Furthermore, the method relies completely upon the results from only three samples obtained during an interval of only 30 minutes just prior to dosing. Just from a consideration of randomness alone, the influence of the average of these three concentrations could be significant. More troubling than the small number of observations is the brief time span from which they are taken. It is known that there is a circadian effect on hormone levels, and the Day –1 data from this study clearly confirmed the presence of the circadian effect. Therefore, unless a subject's expected T_4 levels during the 30 minute time frame just prior to dosing happens also to be the expected average for a 24-hour cycle, the corrected AUC by this method is in error.

Correction Method 2 depends upon the assumption that endogenous production of levothyroxine is completely suppressed when a single large dose of exogenous levothyroxine sodium is given to healthy subjects. Therefore, already available endogenous levothyroxine will decline at rate defined by its half-life, which is assumed to be 7 days. This method also has several undesirable characteristics. Method 2 gives a reasonable correction only if production of endogenous T_4 abruptly and completely stops when study drug is administered and does not resume during the sampling period. Even if this unlikely assumption is true, the correction will be in error for a given subject, with the size of the error depending on how much the given subject's elimination half-life differs from 7 days. The half-life of levothyroxine is not very well documented in healthy volunteers and the 7-day half-life is an approximation based on data from isotope studies with levothyroxine. As previously noted, TSH levels after dosing with levothyroxine sodium were definitely suppressed, but not completely. Thus, it seems very unlikely that endogenous T_4 production would be reduced to zero, with an accompanying 7-day half-life. The use of a single value for levothyroxine half-life for all healthy subjects (regardless of gender, race, and age) at all times is clearly a significant oversimplification. However, estimation of a levothyroxine half-life for each subject in each period is not possible using the currently recommended design in healthy volunteers. Moreover, as with Method 1, Method 2 relies heavily on the average of three concentrations taken immediately before dosing. In particular, for the case in which a subject randomly has a pre-dose average considerably higher than typical for that subject, the corrected AUC is more likely to be negative.

The third method of baseline correction (Method 3) employed in this study corrected the T_4 concentration at each time of post-dose sampling by the corresponding baseline T_4 concentration observed at the same time of day during the 24-hour period preceding the dose, *i.e.*, on Study Day –1. This method provides some advantages in comparison to Methods 1 and 2. The obvious advantages for this method are a) it does not rely on just three samples collected over a very short time period prior to dosing for the correction, and b) the post-dose T_4 concentration is adjusted based on the actual baseline T_4 concentration at the same clock time of the day before dosing in the same subject in the same period, and thus, this method takes into account the diurnal variation in the baseline

T_4 concentration throughout the day in each subject, which is ignored by Methods 1 and 2.

In contrast to Method 2, for Method 3, endogenous T_4 production is not assumed to abruptly stop following study drug administration and a constant value for the elimination half-life across subjects is not assumed. However, similar to Method 1, Method 3 relies on the assumption that there is no suppression of endogenous production when a single dose of exogenous levothyroxine sodium is given to healthy volunteers. Furthermore, Method 3 requires the assumption that the circadian pattern in the endogenous T_4 production does not change when a single large dose of exogenous levothyroxine is administered to healthy subjects.

The impact of administration of large doses of levothyroxine sodium (*e.g.*, 600 µg) on the endogenous production of T_4 is not known. However, the TSH levels are clearly, but not completely, suppressed after administration of the large doses of levothyroxine sodium to the healthy volunteers in this study. The large exogenous dose may also affect the clearance of total T_4 *via* numerous feedback mechanisms. The TSH serum concentration-time data provide clear evidence of the limitations for each of the three methods of correction utilized in this study. Method 2 assumes that endogenous T_4 production is abruptly and completely stopped after study drug administration while Methods 1 and 3 assume that there is no suppression of endogenous production when a single dose of exogenous levothyroxine sodium is given to healthy volunteers.

The FDA Guidance[2] recommended a minimum 35-day washout period between the doses of levothyroxine sodium to minimize carryover. The 24-hour profiles of the baseline T_4 serum concentrations on the day before dosing were clearly not the same for the three study periods even though the washout periods between the doses of levothyroxine sodium in this study were 44 days between Periods 1 and 2 and 53 days between Periods 2 and 3. The Day −1 baseline T_4 data from this study provide convincing evidence that there are carryover effects from the successive study doses, even from the Period 1 dose to the Period 3 dose, and that the carryover effects of the dose levels differ. Carryover effect from the 600 µg dose resulted in higher T_4 levels than carryover effects of the two lower doses. Exploratory analyses of post-dose uncorrected C_{max} and AUC give additional strong evidence of these carryover effects. Also, such unequal carryover effects are present for C_{max} with all three methods of correction. Another component of the period effect may be the presence of seasonal and annual variations in hypothalamic-pituitary-thyroid hormone concentrations in humans. Significant seasonal and annual rhythms in serum TSH and T_3 levels have been reported in the literature.[4] However, the amplitude of the circannual rhythm is probably not as large as that of the daily circadian variation.[4] Therefore, the results from our studies suggest that a much longer washout period between dosing would be required to truly reduce the impact of carryover between dosing periods.

The results of this study strongly suggest that obtaining additional blood samples on Study Day –1 provided data that improved the method of correction for endogenous levels of T_4, accounting for the possibility of a circadian pattern. Additional samples during the afternoon and night hours on the day before dosing and on the days after dosing may provide further benefits to this method of correcting for the endogenous baseline.

It is widely recognized that dose initiation and titration need to be done in susceptible groups with the 12.5 µg dosage strength. In the package insert of levothyroxine sodium products,[5] it states under 'DOSAGE AND ADMINISTRATION – Specific Patient Populations' "the recommended starting dose of levothyroxine sodium in elderly patients with cardiac disease is 12.5 – 25 µg/day, with gradual dose increments at 4 to 6 week intervals. The levothyroxine sodium dose is generally adjusted in 12.5 to 25 µg increments until the patient with primary hypothyroidism is clinically euthyroid and the serum TSH has normalized." NDA approved levothyroxine sodium tablets are available in strengths that differ from their nearest doses by 12 to 13 µg/tablet: that is 75, 88, 100, 112, 125, 137 and 150 µg tablet strengths. The 88 and 112 µg strengths are 12% less or greater, respectively, than the 100 µg strength.

Even though the three methods of correction for endogenous T_4 baseline improve the ability to distinguish between products that are truly different in dose by 25% to 33%, none of the three correction methods were able to distinguish between two products that differ by 12.5%. As stated earlier and similar to the findings with the uncorrected data, the 450 µg dose would continue to be declared bioequivalent to the 400 µg dose after correcting for endogenous T_4 baseline using any of the three correction methods. Narrowing the 90% confidence intervals for evaluating bioequivalence after correcting for endogenous T_4 baseline from the standard range of 0.80 to 1.25 would reduce the chance that two products that differ by 12.5% would be declared bioequivalent.

The potential for conducting bioequivalence trials in athyreotic subjects, a model that minimizes confounding effects from endogenous T_4 due to the absence of residual endogenous hormone, must also be considered. A study in athyreotic subjects would presumably be a multiple-dose study and long enough to properly address the issue of carryover effect. Such a study in athyreotic subjects would utilize therapeutic doses of levothyroxine sodium and remove the need for a method of baseline correction.

Conclusions

This study illustrates some important flaws in the design and analysis of single-dose crossover studies in healthy volunteers to assess bioequivalence of levothyroxine sodium products, stemming from the significant and complex contribution of endogenous T_4. First, the results indicate that the use of baseline uncorrected T_4 C_{max}, AUC_{48}, AUC_{72} and AUC_{96} values would result in declaring two products bioequivalent when they actually differ by as much as 25% to 33% (450 µg and 400 µg *versus* 600 µg). The 450 µg dose and the 400 µg dose would both be declared bioequivalent to the 600 µg dose because the 90% confidence intervals for evaluating bioequivalence without correction for endogenous T_4 baseline were contained within the 0.80 to 1.25 range. Considering the margin by which the conditions for declaring bioequivalence were passed in this study, products that differ by even more than 33% would also have a high likelihood of being declared bioequivalent.

Second, the results from this study indicate that the use of baseline corrected C_{max}, AUC_{48}, AUC_{72} and AUC_{96} values would reduce the likelihood that two products would be declared bioequivalent when they actually differ by 25% to 33%. After correcting for endogenous T_4 levels using each of the three correction methods employed in this study, neither the 450 µg dose nor the 400 µg dose would be declared bioequivalent to the 600 µg dose because the 90% confidence intervals for evaluating bioequivalence were not contained within the 0.80 to 1.25 range for C_{max}, AUC_{48}, AUC_{72} and AUC_{96}.

Third, the 450 µg dose would continue to be declared bioequivalent to the 400 µg dose utilizing the C_{max}, AUC_{48}, and AUC_{96} values for the baseline corrected T_4 data by any of the three methods of correction. A 12.5% difference (400 µg *versus* 450 µg) in levothyroxine sodium products may have a clinically relevant adverse impact on patients. Thus, it is apparent that simple methods of correction for endogenous T_4 concentrations in healthy volunteers are inadequate since these concentrations not only fluctuate on a diurnal cycle but may also be differentially affected by products with different rates and extents of absorption. Additionally, there is evidence of significant carryover from one dosing period to subsequent periods even with washout periods up to 53 days.

The potential for conducting multiple-dose bioequivalence trials in athyreotic subjects, a model that minimizes confounding effects from endogenous T_4 due to the absence of residual endogenous hormone, must also be considered. Such a study in athyreotic subjects would utilize therapeutic doses of levothyroxine sodium and remove the need for a method of baseline correction.

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